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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
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<input type="checkbox"/> Additional inventors are being named on the ___ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
DIFFERENTIALLY EXPRESSED IN SQUAMOUS CELL CARCINOMA GENE-1 (DESC1)					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input type="checkbox"/> Customer Number		<input type="text"/>		Place Customer Number Bar Code Label here	
OR		Type Customer Number here			
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		74		<input type="checkbox"/> Small Entity Statement	
<input type="checkbox"/> Drawing(s) Number of Sheets				<input checked="" type="checkbox"/> Other (specify) <b>FIGURES (3)</b>	
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees				FILING FEE AMOUNT (\$)	
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input type="checkbox"/> No.					
<input checked="" type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: <b>NATIONAL INSTITUTES OF HEALTH # CA63134, # DE12704</b>					

Respectfully submitted,

SIGNATURE

*James C Lang*

Date

2 / 26 / 99

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REGISTRATION NO.

(if appropriate)

Docket Number:

LANG-1

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**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C., 20231.

# **Differentially Expressed in Squamous Cell Carcinoma Gene-1 (DESC1)**

## ***Government Interests***

**This invention was made in part with government support under CA63134 and DE12704 awarded by the National Institutes of Health. The U.S. Government has certain rights in the invention.**

## ***Field of the Invention***

The present invention relates to a novel human gene encoding a polypeptide which is a member of the serine protease gene family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Differentially Expressed in Squamous Cell Carcinoma Gene-1, or "DESC1." This invention also relates to DESC1 polypeptides, as well as vectors, host cells, antibodies directed to DESC1 polypeptides, and the recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders related to cancer of the head and neck and to cancers of epithelial cell origin at all other sites, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of activity.

## ***Background of the Invention***

Investigation of the genetic alterations underlying squamous cell carcinoma has recently allowed identification of a number of key genes demonstrating consistent aberrations during disease progression. Studies of loss of heterozygosity in tumor tissue has implicated alterations to a large number of additional chromosomal loci in tumor development for which no known genetic alterations have been identified. Therefore, there exists a need for identification and characterization of the novel genes located at the site of such chromosomal aberrations since manipulation of these genes and the polypeptides they encode may play a role in detecting, preventing, ameliorating or curing cancer.

In order to identify novel genes associated with the development of cancer, tumor tissue taken from a squamous cell carcinoma-positive neck node and matched normal tissue was subject to Representational Difference Analysis (RDA), to allow cloning of

genes present in the normal tissue but absent from tumor tissue. The rationale is that such genes may represent tumor suppressor genes or markers for disease progression. DESC1 was cloned by RDA.

### ***Summary of the Invention***

The present invention relates to a novel polynucleotide and the encoded polypeptide of DESC1. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders relates to the polypeptides, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying binding partners of DESC1.

### ***Brief Description of the Drawings***

Figure 1 shows the nucleotide sequence (SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35) and the deduced amino acid sequence (SEQ ID NO:2) of DESC1. The predicted hydrophobic transmembrane sequence or signal peptide is located at about amino acids 18-35 and is underlined.

Figure 2 shows the regions of identity between the amino acid sequence of the DESC1 protein and the translation product of Human Airway Trypsin-like protease (Yamaoka et al., J. Biol. Chem., 273:11895-11901, 1998) determined by BLAST analysis. Identical amino acids between the two polypeptides are boxed. By examining amino acid identity, the skilled artisan can readily identify conserved domains between the two polypeptides.

Figure 3 shows an analysis of the DESC1 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the DESC1 protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these graphs are contemplated by the present invention and are shown in Table1.

## *Detailed Description*

### Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

The term "isolated" does not refer to whole natural chromosomes (e.g., chromosome spreads), genomic or cDNA libraries, whole cell total RNA or mRNA preparations, genomic DNA digests (including those gel separated by electrophoresis), sheared whole cell genomic DNA preparations, or other compositions where the art demonstrates no distinguishing features of the polynucleotide sequences of the present invention.

In specific embodiments, the polynucleotides of the invention are less than 300kb, 200kb, 100kb, 50kb, 15kb, 10kb, 7.5kb, 5.0kb, 4.0kb, 3.0kb, 2.5kb, 2.0kb, 1.5kb, 1.0kb, or 0.5kb in length. In a further embodiment, polynucleotides of the invention do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the DESC1 gene in the genome).

In the present invention, a "secreted" DESC1 protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as a DESC1 protein released into the extracellular space without necessarily containing a signal sequence. If the DESC1 secreted protein is released into the extracellular space, the DESC1 secreted protein can undergo extracellular processing to produce a "mature" DESC1 protein. For example, an extracellular, membrane-bound form may be further proteolytically processed to generate a soluble form. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, a DESC1 "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35, or a nucleic acid sequence encoding an amino acid sequence contained in SEQ ID NO: 2 or recombinant DESC1 clones C3, C6, C12 and C35. For example, the DESC1 polynucleotide can contain the nucleotide sequence of the full length cDNA

sequence, including the 5' and 3' untranslated sequences, the coding region, with or without a signal sequence, a soluble polypeptide or secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a DESC1 "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length DESC1 sequence identified as SEQ ID NO:1 was generated by overlapping sequences from recombinant clones C3, C6, C12 and C35 (contig analysis).

A DESC1 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35, the complement thereof, or the cDNA within the deposited clone. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the DESC1 polynucleotides at moderately high stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M  $\text{NaH}_2\text{PO}_4$ ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of

"polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The DESC1 polynucleotide can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, DESC1 polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the DESC1 polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. DESC1 polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

DESC1 polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The DESC1 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the DESC1 polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given DESC1 polypeptide. Also, a given DESC1 polypeptide may contain many types of modifications. DESC1 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic DESC1 polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation,

GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, Proteins-Structure and Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35" refers to a DESC1 polynucleotide sequence while "SEQ ID NO:2" refers to a DESC1 polypeptide sequence.

A DESC1 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a DESC1 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the DESC1 polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the DESC1 polypeptide (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the DESC1 polypeptide.) "Biological activity" of a DESC1 polypeptide includes, but is not limited to:

- (a) Activity as a protease, preferably a serine protease
- (b) Activity as a polypeptide capable of increasing or decreasing the proliferation rate of eukaryotic or procaryotic cells
- (c) Activity as a polypeptide capable of inducing the differentiation or dedifferentiation of eukaryotic cells
- (d) Activity as a polypeptide capable of increasing or decreasing the death rate of eukaryotic or procaryotic cells preferably by altering the biological processes of programmed cell death or apoptosis or necrosis
- (e) Activity as a polypeptide capable of altering the rate of cellular senescence
- (f) Activity as a polypeptide capable of altering a signal transduction pathway within a cell

#### **DESC1 Polynucleotides and Polypeptides**

DESC1 recombinant clones C3, C6, C12 and C35 were isolated from normal human epithelial tissue. These clones comprise the entire coding region identified as SEQ ID NO:2. Recombinant clones C3, C6, C12 and C35 comprise a contiguous



sequence of 1461 nucleotides (SEQ ID NO:1), which encodes a predicted open reading frame of 422 or 423 amino acid residues (Figure 1 and SEQ ID NO:2). The open reading frame begins at a N-terminal methionine located at nucleotide position 53 or 56, and ends at a stop codon at nucleotide position 1322. The predicted molecular weight of the DESC1 protein should be about 44 kDa.

Subsequent Northern analysis also showed DESC1 expression in oral epithelium, epithelium of the oropharynx, pharynx and larynx, prostate and testes, a pattern consistent with epithelial specific expression. Subsequent analysis by RT-PCR also showed expression in skin, prostate, testes and cell lines of epithelial origin, specifically adult and neonatal normal keratinocytes, normal prostate epithelial cells and squamous cell carcinoma cell lines SCC9 and SCC25. Expression is highest in skin and tissue from the head and neck. Lower expression is seen in prostate and testes. Two primary transcripts of approximately 2.2 kb and 1.6 kb are observed in head and neck tissue and prostate. A single 1.6 kb transcript is seen in testes and an additional transcript of approximately 4.4kb is observed exclusively in prostate tissue. Included in the present invention are therefore polynucleotides comprising alternative transcripts of the DESC1 gene and the polypeptides encoded thereby.

Using BLAST analysis, SEQ ID NO:2 was found to be homologous to members of the serine protease gene family. Particularly, SEQ ID NO:2 contains domains homologous to the translation product of the mRNA for Human Airway Trypsin-like protease (Figure 2). Included in the present invention are polypeptide sequences comprising the following conserved domains: (a) a predicted hydrophobic transmembrane domain located at about amino acids 18-35; (b) a predicted catalytic cleavage site located at about amino acids 190-191; and (c) a predicted catalytic domain located at about amino acids 191-422, containing conserved residues comprising the serine protease catalytic triad at about amino acids 231 (histidine), 276 (aspartic acid) and 372 (serine). These polypeptide fragments of DESC1 and polynucleotides encoding said fragments are specifically contemplated in the present invention. Because homologous domains exist between DESC1 and other members of the serine protease gene family, and because these domains comprise the important functional domains of the catalytic cleavage site, the catalytic triad and the hydrophobic transmembrane domain, the data suggests that DESC1 may also be a transmembrane serine protease.

As shown in Figure 1, the predicted mature protein encompasses about 422 amino acids, while the predicted secreted and cleaved form of DESC1, which may be membrane-bound or soluble, encompasses about 232 amino acids (residues 191-422). These polypeptide fragments of DESC1 are specifically contemplated in the present invention.

SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 and the translated SEQ ID NO:2 are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 may be used to generate antibodies which bind specifically to DESC1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides the generated nucleotide sequence identified as SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 and the predicted translated amino acid sequence identified as SEQ ID NO:2. The gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the DESC1 gene from appropriate sources of genomic material.

Also provided in the present invention are species homologs of DESC1. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

The DESC1 polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The DESC1 polypeptides may be in the form of a membrane-bound or soluble secreted protein, including the mature form, or may be a part of a larger protein, such as

a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

DESC1 polypeptides are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a DESC1 polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). DESC1 polypeptides also can be purified from natural or recombinant sources using antibodies of the invention raised against the protein in methods which are well known in the art.

### **Polynucleotide and Polypeptide Variants**

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the DESC1 polynucleotide or polypeptide.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the DESC1 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35, the ORF (open reading frame), or any fragment specified as described herein.

Other methods of determining and defining whether any particular nucleic acid molecule or polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245.) In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result

of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except

that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Other methods for determining and defining whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences shown in SEQ ID NO:2 or to the amino acid sequence encoded by deposited DNA clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the

subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The DESC1 variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. DESC1 polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring DESC1 variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants, which are included in the present invention, can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the

DESC1 polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[most of the molecule could be altered with little effect on either binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes DESC1 polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where

substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of DESC1 include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, DESC1 polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).) DESC1 polypeptide variants, comprising, for example,



amino acid substitutions in the substrate specificity pocket, (Brandon and Tooze, Introduction to Protein Structure, Garland Publishing Inc., New York and London, pgs 231 -246, 1991, and references therein) may also increase or decrease the rate of catalysis, allowing the enzyme to cleave its substrate with greater or lesser efficiency, or may alter the substrate specificity of the DESC1 polypeptide.

### **Polynucleotide and Polypeptide Fragments**

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence shown in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. The short nucleotide fragments are preferably at least 15 nt, at least 20 nt, at least 30 nt, at least 40 nt, at least 50 nt, at least 60 nt, at least 70 nt, at least 80 nt, at least 90 nt and at least 100 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in the deposited clone or the nucleotide sequence shown in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500 nucleotides) are preferred.

Moreover, representative examples of DESC1 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, or 1451 to the end of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:2 or encoded by the DESC1 cDNA. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300,

301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, or 422 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted DESC1 protein as well as the mature form. Further preferred polypeptide fragments include the secreted, membrane-bound or soluble DESC1 protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-211, can be deleted from the amino terminus of either the secreted, membrane-bound or soluble DESC1 polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-211, can be deleted from the carboxy terminus of the secreted, membrane-bound or soluble DESC1 protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these DESC1 polypeptide fragments are also preferred.

Particularly, species of N-terminal deletions of the DESC1 polypeptide can be described by the general formula m-422, where m is an integer from 1 to 421 where m corresponds to the position of the amino acid residue identified in SEQ ID NO:2. Moreover, species of C-terminal deletions of the DESC1 polypeptide can also be described by the general formula 1-n, where n is an integer from 2 to 422 where n corresponds to the position of amino acid residue identified in SEQ ID NO:2. For example, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted DESC1 polypeptide.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:2, where n and m are integers as described above and where m-n is a positive integer. All of the species of polypeptide fragments specified by the formulas: m-422, where m is an integer between 1-421; 1-n, where n is an integer between 2-422; and m-n, where m is an integer between 1-421, n is an integer between 2-422, m-n is a positive integer, can be immediately envisaged using the above description and are therefore not individually listed solely for the purpose of not unnecessarily lengthening the specification. Although it is particularly pointed out that lack of the above described species are included in the present invention.

Also preferred are DESC1 polypeptide and polynucleotide fragments characterized by structural or functional domains. Preferred embodiments of the

invention include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. As set out in the Figures, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha and beta amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions, and Jameson-Wolf high antigenic index regions. Polypeptide fragments of SEQ ID NO:2 falling within conserved domains are specifically contemplated by the present invention. (See Figure 3.) Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active DESC1 fragments. Biologically active DESC1 fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the DESC1 polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity including modification of the following activities:

- (a) Activity as a protease, preferably a serine protease
- (b) Activity as a polypeptide capable of increasing or decreasing the proliferation rate of eukaryotic or procaryotic cells
- (c) Activity as a polypeptide capable of inducing the differentiation or dedifferentiation of eukaryotic cells
- (d) Activity as a polypeptide capable of increasing or decreasing the death rate of eukaryotic or procaryotic cells preferably by altering the biological processes of programmed cell death or apoptosis or necrosis
- (e) Activity as a polypeptide capable of altering the rate of cellular senescence
- (f) Activity as a polypeptide capable of altering a signal transduction pathway within a cell

However, many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences may be related to SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is

any integer between 1 to 1461 of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35, b is an integer of 15 to 1461, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35, and where the b is greater than or equal to a + 14.

### **Epitope-Bearing Portions**

In another aspect, the invention provides peptides and polypeptides comprising epitope-bearing portions of the polypeptides of the present invention. These epitopes are immunogenic or antigenic epitopes of the polypeptides of the present invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response *in vivo* when the whole polypeptide of the present invention, or fragment thereof, is the immunogen. On the other hand, a region of a polypeptide to which an antibody can bind is defined as an "antigenic determinant" or "antigenic epitope." The number of *in vivo* immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. *See, e.g.*, Geysen, et al. (1983) Proc. Natl. Acad. Sci. USA 81:3998- 4002. However, antibodies can be made to any antigenic epitope, regardless of whether it is an immunogenic epitope, by using methods such as phage display. *See e.g.*, Petersen G. et al. (1995) Mol. Gen. Genet. 249:425-431. Therefore, included in the present invention are both immunogenic epitopes and antigenic epitopes.

A list of exemplified amino acid sequences comprising immunogenic epitopes are shown in Table 1 below. It is pointed out that Table 1 only lists amino acid residues comprising epitopes predicted to have the highest degree of antigenicity using the algorithm of Jameson and Wolf, (1988) Comp. Appl. Biosci. 4:181-186 (said references incorporated by reference in their entireties). The Jameson-Wolf antigenic analysis was performed using the computer program PROTEAN, using default parameters (Version 3.11 for the Power MacIntosh, DNASTAR, Inc., 1228 South Park Street Madison, WI). Table 1 and portions of polypeptides not listed in Table 1 are not considered non-immunogenic. The immunogenic epitopes of Table 1 is an exemplified list, not an exhaustive list, because other immunogenic epitopes are merely not recognized as such by the particular algorithm used. Amino acid residues comprising other immunogenic epitopes may be routinely determined using algorithms similar to the Jameson-Wolf analysis or by *in vivo* testing for an antigenic response using methods known in the art. *See, e.g.*, Geysen et al., *supra*; U.S. Patents 4,708,781; 5,194,392; 4,433,092; and 5,480,971 (said references incorporated by reference in their entireties).

It is particularly pointed out that the amino acid sequences of Table 1 comprise immunogenic epitopes. Table 1 lists only the critical residues of immunogenic epitopes

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determined by the Jameson-Wolf analysis. Thus, additional flanking residues on either the N-terminal, C-terminal, or both N- and C-terminal ends may be added to the sequences of Table 1 to generate an epitope-bearing polypeptide of the present invention. Therefore, the immunogenic epitopes of Table 1 may include additional N-terminal or C-terminal amino acid residues. The additional flanking amino acid residues may be contiguous flanking N-terminal and/or C-terminal sequences from the polypeptides of the present invention, heterologous polypeptide sequences, or may include both contiguous flanking sequences from the polypeptides of the present invention and heterologous polypeptide sequences. Polypeptides of the present invention comprising immunogenic or antigenic epitopes are at least 7 amino acids residues in length. "At least" means that a polypeptide of the present invention comprising an immunogenic or antigenic epitope may be 7 amino acid residues in length or any integer between 7 amino acids and the number of amino acid residues of the full length polypeptides of the invention. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. However, it is pointed out that each and every integer between 7 and the number of amino acid residues of the full length polypeptide are included in the present invention.

The immuno and antigenic epitope-bearing fragments may be specified by either the number of contiguous amino acid residues, as described above, or further specified by N-terminal and C-terminal positions of these fragments on the amino acid sequence of SEQ ID NO:2. Every combination of a N-terminal and C-terminal position that a fragment of, for example, at least 7 or at least 15 contiguous amino acid residues in length could occupy on the amino acid sequence of SEQ ID NO:2 is included in the invention. Again, "at least 7 contiguous amino acid residues in length" means 7 amino acid residues in length or any integer between 7 amino acids and the number of amino acid residues of the full length polypeptide of the present invention. Specifically, each and every integer between 7 and the number of amino acid residues of the full length polypeptide are included in the present invention.

Immunogenic and antigenic epitope-bearing polypeptides of the invention are useful, for example, to make antibodies which specifically bind the polypeptides of the invention, and in immunoassays to detect the polypeptides of the present invention. The antibodies are useful, for example, in affinity purification of the polypeptides of the present invention. The antibodies may also routinely be used in a variety of qualitative or quantitative immunoassays, specifically for the polypeptides of the present invention using methods known in the art. *See, e.g.,* Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press; 2nd Ed. 1988).

66122747 "663655  
559399 7422799

The epitope-bearing polypeptides of the present invention may be produced by any conventional means for making polypeptides including synthetic and recombinant methods known in the art. For instance, epitope-bearing peptides may be synthesized using known methods of chemical synthesis. For instance, Houghten has described a simple method for the synthesis of large numbers of peptides, such as 10-20 mgs of 248 individual and distinct 13 residue peptides representing single amino acid variants of a segment of the HA1 polypeptide, all of which were prepared and characterized (by ELISA-type binding studies) in less than four weeks (Houghten, R. A. Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985)). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten and coworkers (1986). In this procedure the individual resins for the solid-phase synthesis of various peptides are contained in separate solvent-permeable packets, enabling the optimal use of the many identical repetitive steps involved in solid-phase methods. A completely manual procedure allows 500-1000 or more syntheses to be conducted simultaneously (Houghten et al. (1985) Proc. Natl. Acad. Sci. 82:5131-5135 at 5134).

Epitope-bearing polypeptides of the present invention are used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe, et al., *supra*; Wilson, et al., *supra*, and Bittle, et al. (1985) J. Gen. Virol. 66:2347-2354. If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as -maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µgs of peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to heterologous polypeptide sequences. For example, the polypeptides of the present

invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, any combination thereof including both entire domains and portions thereof) resulting in chimeric polypeptides. These fusion proteins facilitate purification, and show an increased half-life *in vivo*. This has been shown, *e.g.*, for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. *See, e.g.*, EPA 0,394,827; Traunecker et al. (1988) Nature 331:84-86. Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion can also be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. *See, e.g.*, Fountoulakis et al. (1995) J. Biochem. 270:3958-3964. Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag to aid in detection and purification of the expressed polypeptide.

**Table 1**

**Polypeptide fragments of DESC1 comprising immunogenic epitopes:**

**Position of Amino Acids Residues**

Lys 92 to Lys 101  
Phe 107 to Gly 113  
Phe 124 to Lys 135  
Val 149 to Leu 173  
Gly 150 to His 157  
Asn 165 to Leu 173  
Cys 177 to Leu 185  
Thr 195 to Glu 201  
Trp 210 to Cys 216  
Tyr 236 to Trp 242  
Lys 251 to Gly 258  
Glu 266 to Tyr 275  
Asp 299 to Asp 308  
Leu 318 to His 327  
Thr 339 to Tyr 347  
Leu 361 to Gly 374  
Leu 361 to Gln 369  
Asp 366 to Gly 374  
Val 377 to Asp 383  
Ser 392 to Pro 403  
Cys 397 to Lys 402



## Antibodies

The present invention further relates to antibodies and T-cell antigen receptors (TCR) which specifically bind the DESC1 polypeptides of the present invention. The antibodies of the present invention include IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. As used herein, the term "antibody" (Ab) is meant to include whole antibodies, including single-chain whole antibodies, and antigen-binding fragments thereof. Most preferably the antibodies are human antigen binding antibody fragments of the present invention include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V<sub>L</sub> or V<sub>H</sub> domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are any combinations of variable region(s) and hinge region, CH1, CH2, and CH3 domains. The present invention further includes chimeric, humanized, and human monoclonal and polyclonal antibodies which specifically bind the polypeptides of the present invention. The present invention further includes antibodies which are anti-idiotypic to the antibodies of the present invention.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, A. et al. (1991) J. Immunol. 147:60-69; US Patents 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; Kostelny, S.A. et al. (1992) J. Immunol. 148:1547-1553.

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which are recognized or specifically bound by the antibody. The epitope(s) or polypeptide portion(s) may be specified as described herein, *e.g.*, by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be

excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of the polypeptides of the present invention are included. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies which only bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Antibodies of the present invention have uses that include, but are not limited to, methods known in the art to purify, detect, and target the polypeptides of the present invention including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. *See, e.g.*, Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference in the entirety).

The antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. *See, e.g.*, WO 92/08495; WO 91/14438; WO 89/12624; US Patent 5,314,995; and EP 0 396 387.

The antibodies of the present invention may be prepared by any suitable method known in the art. For example, a polypeptide of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. Monoclonal antibodies can be prepared using a wide of techniques known in the art including the use of hybridoma and recombinant

technology. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties).

Fab and F(ab')<sub>2</sub> fragments may be produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments).

Alternatively, antibodies of the present invention can be produced through the application of recombinant DNA technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman U. et al. (1995) *J. Immunol. Methods* 182:41-50; Ames, R.S. et al. (1995) *J. Immunol. Methods* 184:177-186; Kettleborough, C.A. et al. (1994) *Eur. J. Immunol.* 24:952-958; Persic, L. et al. (1997) *Gene* 187 9-18; Burton, D.R. et al. (1994) *Advances in Immunology* 57:191-280; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patents 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743 (said references incorporated by reference in their entireties).

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax, R.L. et al. (1992) *BioTechniques* 12(6):864-869; and Sawai, H. et al. (1995) *AJRI* 34:26-34; and Better, M. et al. (1988) *Science* 240:1041-1043 (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and

antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al. (1991) *Methods in Enzymology* 203:46-88; Shu, L. et al. (1993) *PNAS* 90:7995-7999; and Skerra, A. et al. (1988) *Science* 240:1038-1040. For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. *See e.g.*, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies, S.D. et al. (1989) *J. Immunol. Methods* 125:191-202; and US Patent 5,807,715. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; US Patent 5,530,101; and 5,585,089), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E.A., (1991) *Molecular Immunology* 28(4/5):489-498; Studnicka G.M. et al. (1994) *Protein Engineering* 7(6):805-814; Roguska M.A. et al. (1994) *PNAS* 91:969-973), and chain shuffling (US Patent 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods described above. *See also*, US Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; and WO 98/46645 (said references incorporated by reference in their entireties).

Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide of the present invention. The antibodies may be specific for antigens other than polypeptides of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *in vitro* immunoassays and purification methods using methods known in the art. *See e.g.*, Harbor et al. *supra* and WO 93/21232; EP 0 439 095; Naramura, M. et al. (1994) *Immunol. Lett.* 39:91-99; US Patent 5,474,981; Gillies, S.O. et al. (1992) *PNAS* 89:1428-1432; Fell, H.P. et al. (1991) *J. Immunol.* 146:2446-2452 (said references incorporated by reference in their entireties).

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for

use in immunoassays using methods known in the art. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See e.g., US Patents 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388, WO 91/06570; Ashkenazi, A. et al. (1991) PNAS 88:10535-10539; Zheng, X.X. et al. (1995) J. Immunol. 154:5590-5600; and Vil, H. et al. (1992) PNAS 89:11337-11341 (said references incorporated by reference in their entireties).

The invention further relates to antibodies which act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also included are receptor-specific antibodies which both prevent ligand binding and receptor activation. Likewise, included are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies which activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. The above antibody agonists can be made using methods known in the art. See e.g., WO 96/40281; US Patent 5,811,097; Deng, B. et al. (1998) Blood 92(6):1981-1988; Chen, Z. et al. (1998) Cancer Res. 58(16):3668-3678; Harrop, J.A. et al. (1998) J. Immunol. 161(4):1786-1794; Zhu, Z. et al. (1998) Cancer Res. 58(15):3209-3214; Yoon, D.Y. et al. (1998) J. Immunol. 160(7):3170-3179; Prat, M. et al. (1998) J. Cell. Sci. 111(Pt2):237-247; Pitard, V. et al. (1997) J. Immunol. Methods 205(2):177-190; Liautard, J. et al. (1997) Cytokine 9(4):233-241; Carlson, N.G. et al. (1997) J. Biol. Chem. 272(17):11295-11301; Taryman, R.E. et al. (1995) Neuron 14(4):755-762; Muller, Y.A. et al. (1998) Structure 6(9):1153-1167; Bartunek, P. et al. (1996) Cytokine 8(1):14-20 (said references incorporated by reference in their entireties).

### Fusion Proteins

Any DESC1 polypeptide can be used to generate fusion proteins. For example, the DESC1 polypeptide, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the DESC1 polypeptide can be used to indirectly detect the second protein by binding to DESC1. Moreover, because secreted proteins target cellular locations based on trafficking signals, the DESC1 polypeptides can be used as a targeting molecule once fused to other proteins.

Examples of domains that can be fused to DESC1 polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the DESC1 polypeptide. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the DESC1 polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the DESC1 polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the DESC1 polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, DESC1 polypeptides, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides as described above. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins,

such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the DESC1 polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of DESC1. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the DESC1 polynucleotides or the polypeptides.

### **Vectors, Host Cells, and Protein Production**

The present invention also relates to vectors containing the DESC1 polynucleotide, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

DESC1 polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DESC1 polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance

for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that DESC1 polypeptides may in fact be expressed by a host cell lacking a recombinant vector.

DESC1 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

DESC1 polypeptides, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the DESC1 polypeptides may be glycosylated or may be non-glycosylated. In addition, DESC1 polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal



methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., DESC1 coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with DESC1 polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous DESC1 polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous DESC1 polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

#### **Uses of DESC1 Polynucleotides**

The DESC1 polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Clone DESC1 represents a novel gene, and has been mapped to chromosome 4. Thus, DESC1 polynucleotides can be used in linkage analysis as a marker for chromosome 4.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human DESC1 gene corresponding to the SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per

day using a single thermal cycler. Moreover, sublocalization of the DESC1 polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the DESC1 polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the DESC1 polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the DESC1 polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the DESC1 polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using DESC1

polynucleotides. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, a DESC1 polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

DESC1 polynucleotides are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. DESC1 offers a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The DESC1 polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The DESC1 polynucleotides can be used as additional DNA markers for RFLP.

The DESC1 polynucleotides can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, DESC1 polynucleotides can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from DESC1 sequences. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

Because DESC1 is found expressed in epithelial tissue, DESC1 polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to DESC1 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, measurement of DESC1 expression levels can be used for diagnosis of a number of disorders of the above tissues or cells, particularly of epithelial tissue, higher or lower levels of DESC1 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" DESC1 gene expression level, i.e., the DESC1 expression level in healthy tissue from an individual not having the system disorder. Examples of disorders that can be treated or detected by DESC1 polynucleotides or polypeptides include, but are not limited to all neoplasms, including hyperproliferative and premalignant tissues of epithelial origin and neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital, including prostate. DESC1 polynucleotides or polypeptides may also be useful as prognostic indicators of disease. Methods of detection of normal and altered forms of DESC1 polynucleotides include, but are not limited to detection by Northern analysis, RNase protection assay, reverse transcription polymerase chain

reaction and single stranded conformation polymorphism analysis (see, for example Lang et al., *Oncogene*, 6:2067-2075, 1991; Kenny JJ et al., *Oncogene*, 14:997-1001, 1997; Lang JC et al., *Laryngoscope*, 108:923-928, 1998 and references therein). Thus altered expression of DESC1 relative to a "standard" may be used to predict clinical outcome for individual patients. Also, altered expression of DESC1 may affect the response of neoplasms to radiation, chemotherapeutic or immunotherapeutic treatment and prior screening of neoplasms for levels of DESC1 expression may be used to aid determination of the therapeutic treatment regimen.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying DESC1 gene expression level in cells or body fluid of an individual; (b) comparing the DESC1 gene expression level with a standard DESC1 gene expression level, whereby an increase or decrease in the assayed DESC1 gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the DESC1 polynucleotides can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### **Uses of DESC1 Polypeptides**

DESC1 polypeptides can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

DESC1 polypeptides can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., et al., *J. Cell. Biol.* 105:3087-3096 (1987).) DESC1 polypeptides can also be assayed by Western analysis (see, for example, Harlow and Lane, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Laboratories, 1988). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging

of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a detection or diagnostic method of a disorder, which involves (a) assaying the expression of DESC1 polypeptide in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed DESC1 polypeptide gene expression level compared to the standard expression level is indicative of a marker for a cell type, cellular condition, or disorder.

DESC1 is a serine protease and can be used to cleave naturally occurring substrate proteins and by amino acid substitutions, to cleave proteins which are substrates of other serine proteases. Thus DESC1 polypeptides can be used to cleave a peptide for usage in microsequencing or for peptide mapping of proteins. DESC1 serine protease activity may be assayed utilizing standard methodologies used to demonstrate the activity of other serine proteases, as described for example by Smyth et al., *J. Biol. Chem.*, 267:24418-24425 and utilizing commercially available serine protease substrates including, Benzoyl-prolyl-phenyl-alanyl-arginine-4-nitranilide acetate; Tosyl-glycyl-prolyl-lysine-4-nitranilide acetate; Carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate and N-Methoxycarbonyl-D-norleucyl-glycyl-L-arginine-4-nitranilide acetate (Boehringer Mannheim). Additionally, assay of serine protease activity of DESC1 can

be utilized to identify inhibitors of DESC1 activity, by addition of known protease inhibitors to the assay, such as alpha2-macroglobulin, 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride and Leupeptin (Boehringer Mannheim).

Moreover, DESC1 polypeptides can be used to treat disease. For example, patients can be administered DESC1 polypeptides in an effort to replace absent or decreased levels of the DESC1 polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to DESC1 polypeptides can also be used to treat disease. For example, administration of an antibody directed to a DESC1 polypeptide can bind and reduce overproduction of the polypeptide or inhibit the activity of a DESC1 polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor). Also, administration of Type I signal peptidases may be used to activate DESC1 by cleavage and removal of the DESC1 signal peptide domain, as described by Dalbey et al Trends Biochem Sci 11:474-478, 1992.

At the very least, the DESC1 polypeptides can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. DESC1 polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a natural or recombinant cell, as a way of assessing transformation of the host cell. Moreover, DESC1 polypeptides can be used to test the following biological activities.

#### **Biological Activities of DESC1**

DESC1 polynucleotides and polypeptides can be used in assays to test for one or more biological activities. If DESC1 polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that DESC1 may be involved in the diseases associated with the biological activity. Therefore, DESC1 could be used to treat the associated disease.

#### **Immune Activity**

DESC1 polypeptides or polynucleotides may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation,

differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, DESC1 polynucleotides or polypeptides can be used as a marker or detector of a particular immune system disease or disorder.

DESC1 polynucleotides or polypeptides may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. DESC1 polypeptides or polynucleotides could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, DESC1 polypeptides or polynucleotides can also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, DESC1 polynucleotides or polypeptides could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, DESC1 polynucleotides or polypeptides that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting, important in the treatment of heart attacks (infarction), strokes, or scarring.

DESC1 polynucleotides or polypeptides may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of DESC1 polypeptides or polynucleotides that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by DESC1 include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid



syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by DESC1 polypeptides or polynucleotides. Moreover, DESC1 can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

DESC1 polynucleotides or polypeptides may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of DESC1 polypeptides or polynucleotides that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, DESC1 polypeptides or polynucleotides may also be used to modulate inflammation. For example, DESC1 polypeptides or polynucleotides may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

#### **Hyperproliferative Disorders**

DESC1 polypeptides or polynucleotides can be used to treat or detect hyperproliferative disorders, including neoplasms. DESC1 polypeptides or polynucleotides may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, DESC1 polypeptides or polynucleotides may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by DESC1 polynucleotides or polypeptides include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital, including prostate.

Similarly, other hyperproliferative disorders can also be treated or detected by DESC1 polynucleotides or polypeptides. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

### **Infectious Disease**

DESC1 polypeptides or polynucleotides can be used to treat or detect infectious agents: For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, DESC1 polypeptides or polynucleotides may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by DESC1 polynucleotides or polypeptides. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Bimaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae

(HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. DESC1 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by DESC1 polynucleotides or polypeptides include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Pasteurellaceae Infections (e.g., Actinobacillus, Haemophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. DESC1 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by DESC1 polynucleotides or polypeptides include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies,

Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. DESC1 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using DESC1 polypeptides or polynucleotides could either be by administering an effective amount of DESC1 polypeptide to the patient, or by removing cells from the patient, supplying the cells with DESC1 polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the DESC1 polypeptide or polynucleotide can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### Regeneration

DESC1 polynucleotides or polypeptides can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, DESC1 polynucleotides or polypeptides may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. DESC1 polynucleotides or polypeptides of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using DESC1 polynucleotides or polypeptides to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from

chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the DESC1 polynucleotides or polypeptides.

### Chemotaxis

DESC1 polynucleotides or polypeptides may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

DESC1 polynucleotides or polypeptides may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. As a chemotactic molecule, DESC1 could also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that DESC1 polynucleotides or polypeptides may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, DESC1 polynucleotides or polypeptides could be used as an inhibitor of chemotaxis.

### Neoplasms

DESC1 polynucleotides are also useful in cancer gene therapy. The goal of cancer gene therapy is to insert a normal gene into cancerous tissue in an effort to cure or slow the progression of the disease. Expression of DESC1 is greatly reduced or absent when normal epithelial cells transform into malignant cells. Thus DESC1 may, for example, be inserted into a vector including but not exclusive to adenovirus, herpes simplex virus, retrovirus, vaccinia virus, human papilloma virus or adeno-associated virus and the recombinant virus generated, used to infect cancer tissue in an attempt to cure or slow the progression of the disease. Expression of DESC1 is also absent in all cases of squamous cell carcinoma metastatic to lymph nodes. Thus recombinant viral vectors expressing DESC1 may also be used to treat metastatic cancer. Similarly, DESC1 polypeptides, or activating antibodies to DESC1, may be employed to bathe cancer tissue as a therapeutic measure. Additionally, DESC1 polynucleotides administered in the form of gene therapy, or DESC1 polypeptides, may be administered prior to or

following surgical procedures, radiation therapy or chemotherapy, as an adjunct form of treatment.

### **Binding Activity**

DESC1 polypeptides may be used to screen for molecules that bind to DESC1 or for molecules to which DESC1 binds. The binding of DESC1 and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of DESC1 or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of DESC1, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which DESC1 binds, or at least, a fragment of the receptor capable of being bound by DESC1 (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express DESC1, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing DESC1 (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either DESC1 or the molecule.

The assay may simply test binding of a candidate compound to DESC1, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to DESC1.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing DESC1, measuring DESC1/molecule activity or binding, and comparing the DESC1/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure DESC1 level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure DESC1 level or activity by either binding, directly or indirectly, to DESC1 or by competing with DESC1 for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a

particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the DESC1/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of DESC1 from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to DESC1 comprising the steps of: (a) incubating a candidate binding compound with DESC1; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with DESC1, (b) assaying a biological activity, and (b) determining if a biological activity of DESC1 has been altered.

#### **Other Activities**

DESC1 polypeptides or polynucleotides may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

DESC1 polypeptides or polynucleotides may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, DESC1 polypeptides or polynucleotides may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

DESC1 polypeptides or polynucleotides may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

DESC1 polypeptides or polynucleotides may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

DESC1 polypeptides or polynucleotides may also be used to treat diseases affecting hair growth, including alopecia due to the presence of DESC1 expression in normal keratinocytes.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

## Examples

### Example 1: Isolation of DESC1 cDNA from Expressing Tissue or Cell Lines

Two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 (i.e., within the region of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35 bounded by the 5' NT and the 3' NT of the clone) are synthesized and used to amplify DESC1 using RT-PCR and RNA isolated from expressing tissue as a template. Reverse transcription of DESC1 RNA is performed according to the manufacturer's protocol (Stratagene, La Jolla, CA). The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu$ l of reaction mixture with 0.5  $\mu$ g of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $MgCl_2$ , 0.01% (w/v) gelatin, 20  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of the gene. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves



a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the gene.

#### **Example 2: Isolation of DESC1 Genomic Clones**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35., according to the method described in Example 1.

#### **Example 3: Tissue Distribution of Polypeptides**

Tissue distribution of mRNA expression of is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a probe produced by the method described in Example 1 is labeled with P<sup>32</sup> using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

#### **Example 4: Chromosomal Mapping of DESC1**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5

minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

#### **Example 5: Bacterial Expression of DESC1**

A polynucleotide encoding a DESC1 polypeptide invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

Specifically, to clone the mature/extracellular domain of the DESC1 protein in a bacterial vector, the 5' primer has the sequence 5' insert sequence 3' (SEQ ID NO: 14) containing a Bam HI restriction site followed by a number of nucleotides of the amino terminal coding sequence of the extracellular/mature sequence in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete DESC1 protein shorter or longer than the mature form/extracellular domain of the protein. The 3' primer has the sequence 5' insert sequence 3' containing an Xba I restriction site followed by a number nucleotides complementary to the 3' end of the coding sequence of the DNA sequence of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide, called pHE4a. (ATCC Accession Number 209645, deposited February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from

pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### **Example 6: Purification of DESC1 Polypeptide from an Inclusion Body**

The following alternative method can be used to purify polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16  $\mu$ m membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu$ g of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

#### **Example 7: Cloning and Expression of DESC1 in a Baculovirus Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert polynucleotide into a baculovirus to express. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence of DESC1, including the AUG initiation codon and any naturally associated leader sequence, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

More specifically, the cDNA sequence encoding the full length protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate

containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of  $^{35}\text{S}$ -methionine and 5 uCi  $^{35}\text{S}$ -cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

#### **Example 8: Expression of DESC1 in Mammalian Cells**

DESC1 polypeptide can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the

termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2DHFR (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

#### **Example 9: Construction of N-Terminal and/or C-Terminal DESC1 Deletion Mutants**

The following general approach may be used to clone a N-terminal or C-terminal deletion mutant. Generally, two oligonucleotide primers of about 15-25 nucleotides are derived from the desired 5' and 3' positions of a polynucleotide of SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. The 5' and 3' positions of the primers are determined based on the desired polynucleotide fragment. An initiation and stop codon are added to the 5' and 3' primers respectively, if necessary, to express the polypeptide fragment encoded by the polynucleotide fragment. Preferred polynucleotide fragments are those encoding the N-terminal and C-terminal deletion mutants disclosed above in the "Polynucleotide and Polypeptide Fragments" section of the Specification.

Additional nucleotides containing restriction sites to facilitate cloning of the polynucleotide fragment in a desired vector may also be added to the 5' and 3' primer sequences. The polynucleotide fragment is amplified from genomic DNA or from the deposited cDNA clone using the appropriate PCR oligonucleotide primers and conditions discussed herein or known in the art. The polypeptide fragments encoded by the polynucleotide fragments of the present invention may be expressed and purified in the same general manner as the full length polypeptides, although routine



modifications may be necessary due to the differences in chemical and physical properties between a particular fragment and full length polypeptide.

#### **Example 10: Protein Fusions of DESC1**

Fusion proteins can be used for a variety of applications. For example, fusion of DESC1 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (Traunecker, et al., Nature 331:84-86 1988). Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

```
GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCCACCGTGCC
CAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAACC
CAAGGACACCTCATGATCTCCCGGACTCCTGAGGTACATGCGTGGTGGT
GGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG
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AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCC  
 ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT  
 GTACACCTTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCT  
 GACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGA  
 GAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGG  
 ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA  
 GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGC  
 ACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGC  
 GACGGCCGCGACTCTAGAGGAT

#### **Example 11: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether or a molecule induced by is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10%

Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 12, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2+</sub> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initiate the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### **Example 12: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 12, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants

obtained in Example 12 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by or a molecule induced by .

### **Example 13: Method of Determining Alterations in the DESC1 Gene**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1 or recombinant DESC1 clones C3, C6, C12 and C35. Suggested PCR conditions consist of 35 cycles at 95 degree C for 30 seconds; 60-120 seconds at 52-58 degree C; and 60-120 seconds at 70 degree C, using buffer solutions described in Sidransky, D., et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons of is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in is then cloned and sequenced to validate the results of the direct sequencing.

PCR products of are cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations in not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in the gene. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson, Cg. et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region of (hybridized by the probe) are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

**Example 14: Method of Detecting Abnormal Levels of DESC1 in a Biological Sample**

DESC1 polypeptides can be detected in a biological sample, and if an increased or decreased level of DESC1 is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect DESC1 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to DESC1, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal. The wells are blocked so that non-specific binding of DESC1 to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing DESC1. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbound .

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the in the sample using the standard curve.

### **Example 15: Formulating a Polypeptide**

The DESC1 composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing DESC1 are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

DESC1 is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and R. Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped polypeptides. Liposomes containing the are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. USA 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA

77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, DESC1 is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

DESC1 is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.



DESC1 used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

DESC1 polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, may be employed in conjunction with other therapeutic compounds.

#### **Example 16: Method of Treating Decreased Levels of DESC1**

The present invention also relates to a method for treating an individual in need of an increased level of DESC1 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of DESC1 or an agonist thereof.

Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of DESC1 in an individual can be treated by administering DESC1, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of DESC1 to increase the activity level of DESC1 in such an individual.

For example, a patient with decreased levels of DESC1 polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form.

#### **Example 17: Method of Treating Increased Levels of DESC1**

The present invention relates to a method for treating an individual in need of a decreased level of DESC1 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of DESC1 antagonist. Preferred antagonists for use in the present invention are DESC1-specific antibodies.

Antisense technology is used to inhibit production of DESC1. This technology is one example of a method of decreasing levels of DESC1 polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of DESC1 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated.

#### **Example 18: Method of Treatment Using Gene Therapy - Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing DESC1 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting

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mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector contains properly inserted DESC1.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the DESC1 gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the DESC1 gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

#### **Example 19: Method of Treatment Using Gene Therapy - In Vivo**

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the DESC1 polypeptide. The polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata H. et al. (1997) Cardiovasc. Res. 35(3):470-479, Chao J et al. (1997) Pharmacol. Res. 35(6):517-522, Wolff J.A. (1997) Neuromuscul. Disord. 7(5):314-318, Schwartz B. et al. (1996) Gene Ther. 3(5):405-411, Tsurumi Y. et al. (1996) Circulation 94(12):3281-3290 (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The DESC1 polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked DESC1 polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DESC1 polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips. After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15  $\mu$ m cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DESC1 DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

**Example 20: Use of DESC1 as a Diagnostic or Prognostic Indicator for Squamous Cell Carcinoma**

DESC1 RNA is expressed in normal human epithelial tissue and was cloned from such tissue. DESC1 RNA is also expressed in normal human epidermal keratinocytes undergoing exponential growth in tissue culture (NEHK cells; Clonetics, San Diego, CA). DESC1 RNA expression is reduced or absent in squamous cell carcinoma (SCC). Thus, in one embodiment, reduced DESC1 expression in neoplastic tissue of epithelial origin may be considered to be a diagnostic indicator of SCC. Additionally, due to the presence of DESC1 RNA in exponentially growing keratinocytes, in a second embodiment, DESC1 may also be expressed in hyperproliferative epithelium which has not undergone malignant change. Therefore DESC1 expression may be used to distinguish between tissue exhibiting benign hyperplasia and SCC. In particular, this function may be important for diagnosis of carcinoma of the prostate, since the prostate specific antigen (PSA) test does not readily demonstrate a clear distinction between benign disease and carcinoma. Alternatively, in a third embodiment, DESC1 expression may be lost at a stage earlier than development of carcinoma and may already be lost in tissues exhibiting premalignant changes. In this case, loss of DESC1 expression may be a prognostic indicator of those premalignant lesions, for example, leukoplakic lesions in the oral cavity, which will later progress to carcinoma.

Several methods are available to allow detection of DESC1 in tissue specimens including SCC. A preferred method involves freezing of resected tissue by immersion in liquid nitrogen at the site of surgery in the Operating Room (OR). Resected tissue comprises SCC and matched normal, unaffected epithelial tissue. If available, metastatic lymph node or premalignant tissue is also collected. Frozen tissue samples are provided to the investigator by the Cooperative Human Tissue Network and polynucleotides (in this case, RNA) extracted as described in Gramza et al., BioTechniques, 18:228-229, 1995. Briefly, tissue is crushed in a plastic bag, which has been selected for resistance to shearing at low temperature, Trizol reagent (GIBCO BRL, Gaithersburg, MD) added and RNA extracted. Purified RNA samples are then subject to Reverse Transcription-Polymerase Chain Reaction (RT-PCR) amplification in order to detect expression of DESC1 RNA. RNA (1µg) is reverse transcribed using Superscript II (Gibco BRL, Gaithersburg, MD) in the presence of random hexamer oligonucleotides in a final volume of 20µl, according to the manufacturer's instructions. Reaction conditions are 37°C 45min, ramp to 50°C 15min, 50°C 15min, 90°C 10min. Samples are RNased in the presence of RNase H and 2µl of reverse transcription (RT) reaction (equivalent to

0.1µg of starting RNA) then used for PCR amplification. Primer pairs used for DESC1 PCR amplification are: Sel 10: 5'-CCTGTTCCCTACACAAATGCAGTAC-3' and Sel 11: 5'-TGCATCAAGCAAACAGTTTATTGAG ATC-3', and for control gene hypoxanthine phosphoribosyl transferase (HPRT) 5'-GTAATGACCAGTCAACAG GGGAC-3' and 5'-CCAGCAAGCTTGCGACCTTGACCA-3'. PCR amplification of the HPRT gene was performed as a control to determine integrity of RNA. Primers were designed by computer analysis (Oligo 4.0; NBI, Hamel, MN) of available DNA sequence for each gene and are intron-spanning precluding PCR amplification of any residual DNA present in RNA samples. PCR amplification was performed in the presence of 2 units Taq 2000 DNA polymerase (Stratagene, La Jolla, CA), 100ng each primer, 10µM dNTPs, and 1.5mM MgCl<sub>2</sub> 0.25µCi α[<sup>32</sup>P]dCTP in a final volume of 25µl. Separate reactions are performed for each primer pair with reaction conditions; 96°C for 3min, followed by 94°C 30sec, 55°C 30sec, 72°C 1min (31 cycles HPRT, 34 cycles DESC1) and a final 5min extension at 72°C. PCR samples are then run through 2% agarose gels and presence of amplified product and correct product size (DESC1 490bp and HPRT, 177bp) verified by ethidium bromide fluorescence in the presence of 100bp size markers (Gibco BRL, Gaithersburg, MD). PCR product is then transferred to nylon membrane by electroblotting, the membrane exposed to x-ray film and band intensities for each sample compared by densitometry analysis. Band intensity of DESC1 RT-PCR product is then compared and intensity is directly proportional to the level of expression of DESC1 RNA in the original tissue sample.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Moreover, the sequence listing is herein incorporated by reference.

*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
  - (a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35 at least 20 nucleotides in length;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:2 or the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35 at least 13 amino acid residues in length;
  - (c) a polynucleotide encoding a polypeptide proteolytic domain of SEQ ID NO:2 or the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35;
  - (d) a polynucleotide encoding a polypeptide antigenic epitope of SEQ ID NO:2 or the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:2 or the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35 wherein said polypeptide has biological activity;
  - (f) a polynucleotide which is an allelic variant of SEQ ID NO:1 or the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35;
  - (g) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(f), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.
2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a soluble form of the DESC1 polypeptide.
3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:2 or the coding sequence of the human cDNA included in recombinant DESC1 clones C3, C6, C12 and C35.



4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the human cDNA sequence included in recombinant DESC1 clones C3, C6, C12 and C35.
5. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.
6. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
7. A recombinant host cell produced by the method of claim 6.
8. The recombinant host cell of claim 7 comprising vector sequences.
9. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:
- (a) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in recombinant DESC1 clones C3, C6, C12 and C35 wherein said fragment is at least 13 amino acid residues in length;
  - (b) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in recombinant DESC1 clones C3, C6, C12 and C35 having biological activity;
  - (c) a polypeptide proteolytic domain of SEQ ID NO:2 or the encoded sequence included in recombinant DESC1 clones C3, C6, C12 and C35
  - (d) a polypeptide antigenic epitope of SEQ ID NO:2 or the encoded sequence included in recombinant DESC1 clones C3, C6, C12 and C35
  - (e) a mature form of the human polypeptide DESC1;
  - (f) a full length soluble form of the human polypeptide DESC1;
  - (g) a full-length membrane-bound form of the human polypeptide DESC1;
  - (h) an allelic variant of the polypeptide DESC1 or
  - (i) a species homologue of the polypeptide DESC1.
10. The isolated polypeptide of claim 9, wherein a mature form, a full length secreted protein, or a soluble form comprises sequential amino acid deletions from either the C-terminus or the N-terminus.
11. An isolated antibody that binds specifically to the isolated polypeptide of claim 9.

12. A recombinant host cell that expresses the isolated polypeptide of claim 9.
13. A method of making an isolated polypeptide comprising:
  - (a) culturing a cell that expresses the polypeptide of claim 9; and
  - (b) recovering said polypeptide.
14. The polypeptide produced by claim 13.
15. A method for preventing, treating, or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 9 or of the polynucleotide of claim 1.
16. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a protein comprising:
  - (a) determining the presence or absence of a mutation in the polynucleotide of claim 1;
  - (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.
17. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a protein comprising:
  - (a) determining the presence or amount of expression of the polypeptide of claim 9 or the presence of a mutation in the polypeptide of claim 9 in a biological sample;
  - (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.
18. A method for identifying binding partner to the polypeptide of claim 9 comprising:
  - (a) contacting the polypeptide of claim 9 with a binding partner; and
  - (b) determining whether the binding partner effects an activity of the polypeptide.
19. The isolated gene corresponding to the cDNA sequence of SEQ ID NO:2.



## Abstract

The present invention relates to a novel human protein called DESC1, and isolated polynucleotides encoding this protein. Also provided are vectors, host cells, antibodies, and recombinant methods for producing this human protein. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to this novel human protein.

659355 242347 533555

1 tgacttggatgtagacctcgaccttcacaggactcttcattgctgggttgcaatg ATG TAT CGG CCA GAT GTG 73  
1 M Y R P D V 6

74 GTG AGG GCT AGG AAA AGA GTT TGT TGG GAA CCC TGG GTT ATC GGC CTC GTC ATG TTC ATA 133  
7 V R A R K R V C W E P W V I G L V M F I 26

134 TCC CTG ATT GTC CTG GCA GTG TGC ATT GGA GTC ACT GTT CAT TAT GTG AGA TAT AAT CAA 193  
27 S L I V L A V C I G V T V H Y V R Y N Q 46

194 AAG AAG ACC TAC AAT TAC TAT AGC ACA TTG TCA TTT ACA ACT GAC AAA CTA TAT GCT GAG 253  
47 K K T Y N Y Y S T L S F T T D K L Y A E 66

254 TTT GGC AGA GAG GCT TCT AAC AAT TTT ACA GAA ATG AGC CAG AGA CTT GAA TCA ATG GTG 313  
67 F G R E A S N N F T E M S Q R L E S M V 86

314 AAA AAT GCA TTT TAT AAA TCT CCA TTA AGG GAA GAA TTT GTC AAG TCT CAG GTT ATC AAG 373  
87 K N A F Y K S P L R E E F V K S Q V I K 106

374 TTC AGT CAA CAG AAG CAT GGA GTG TTG GCT CAT ATG CTG TTG ATT TGT AGA TTT CAC TCT 433  
107 F S Q Q K H G V L A H M L L I C R F H S 126

434 ACT S G GAT CCT GAA ACT GTA GAT AAA ATT GTT CAA CTT GTT TTA CAT GAA AAG CTG CAA 493  
127 T E D P E T V D K I V Q L V L H E K L Q 146

494 GAT GCT GTA GGA CCC CCT AAA GTA GAT CCT CAC TCA GTT AAA ATT AAA AAA ATC AAC AAG 553  
147 D A V G P P K V D P H S V K I K K I N K 166

554 ACA GAA ACA GAC AGC TAT CTA AAC CAT TGC TGC GGA ACA CGA AGA AGT AAA ACT CTA GGT 613  
167 T E T D S Y L N H C C G T R R S K T L G 186

614 CAG AGT CTC AGG ATC GTT GGT GGG ACA GAA GTA GAA GAG GGT GAA TGG CCC TGG CAG GCT 673  
187 Q S L R I V G G T E V E E G E W P W Q A 206

674 AGC CTG CAG TGG GAT GGG AGT CAT CGC TGT GGA GCA ACC TTA ATT AAT GCC ACA TGG CTT 733  
207 S L Q W D G S H R C G A T L I N A T W L 226

734 GTG AGT GCT GCT CAC TGT TTT ACA ACA TAT AAG AAC CCT GCC AGA TGG ACT GCT TCC TTT 793  
227 V S A A H C F T T Y K N P A R W T A S F 246

794 GGA GTA ACA ATA AAA CCT TCG AAA ATG AAA CGG GGT CTC CGG AGA ATA ATT GTC CAT GAA 853  
247 G V T I K P S K M K R G L R R I I V H E 266

854 AAA TAC AAA CAC CCA TCA CAT GAC TAT GAT ATT TCT CTT GCA GAG CTT TCT AGC CCT GTT 913  
267 K Y K H P S H D Y D I S L A E L S S P V 286

914 CCC TAC ACA AAT GCA GTA CAT AGA GTT TGT CTC CCT GAT GCA TCC TAT GAG TTT CAA CCA 973  
287 P Y T N A V H R V C L P D A S Y E F Q P 306

974 GGT GAT GTG ATG TTT GTG ACA GGA TTT GGA GCA CTG AAA AAT GAT GGT TAC AGT CAA AAT 1033  
307 G D V M F V T G F G A L K N D G Y S Q N 326

1034 CAT CTT CGA CAA GCA CAG GTG ACT CTC ATA GAC GCT ACA ACT TGC AAT GAA CCT CAA GCT 1093  
327 H L R Q A Q V T L I D A T T C N E P Q A 346

1094 TAC AAT GAC GCC ATA ACT CCT AGA ATG TTA TGT GCT GGC TCC TTA GAA GGA AAA ACA GAT 1153  
347 Y N D A I T P R M L C A G S L E G K T D 366

1154 GCA TGC CAG GGT GAC TCT GGA GGA CCA CTG GTT AGT TCA GAT GCT AGA GAT ATC TGG TAC 1213  
367 A C Q G D S G G P L V S S D A R D I W Y 386

1214 CTT GCT GGA ATA GTG AGC TCG GGA GAT GAA TGT GCG AAA CCC AAC AAG CCT GGT GTT TAT 1273  
387 L A G I V S S G D E C A K P N K P G V Y 406

1274 ACT AGA GTT ACG GCC TTG CGG GAC TGG ATT ACT TCA AAA ACT GGT ATC TAA gagagaaaagcc 1336  
407 T R V T A L R D W I T S K T G I \* 423

1337 tcatggaacagataaacattttttttgttttttgggtgtggaggccatttttagagatacagaattggagaagacttgca 1416

1417 aaacagctagatttgactgatctcaataaactgtttgcttgatgcaaaaaaaaaa 1471

Figure 1

DESC1	1	M Y R R D V V R A R K R V C W E P W V I G L V M F I S L I V L A V C I	35
HAT	1	M Y R P A R V T S T S R F L N P Y V V C F I V V A G V V L - L A V T I	34
DESC1	36	G V T V H Y V R Y N O K K T Y N Y S T L S F T T D K L Y A E F G R E	70
HAT	35	A L L V Y F L A F D O K S - Y F Y R S S F Q L L N V E Y N S Q L N S P	68
DESC1	71	A S N N F T E M S Q R L E S M V K N A F Y K S P L R E E F V K S Q V I	105
HAT	69	A T Q E Y R T L S G R I E S L I T K T R K E S N L R N Q F I R A H V A	103
DESC1	106	K F S Q Q K H G V L A H M L L I C R E H S T E D P E T V D K I V Q L V	140
HAT	104	K L R O D G S G V R A D V V M K F Q F T R N N N G A S M K S R I E S V	138
DESC1	141	L H E K L Q D A V G P P K V D P H S V K I K K I N K T E T D S Y L N H	175
HAT	139	L R Q M L N N S G N L E I N P S T E I T S L T D Q A A A N W L I N E C	173
DESC1	176	C C G T R R S K T L G C S L R I V C G T E V E E S E V P W O A S T Q W	210
HAT	174	G A G P D L I T L S E Q - - R I L G C T E A E E G S W P W Q V S L R L	206
DESC1	211	D G S H R O G A T L I N A T W L V S A A H C F T T Y K N P A R W T A S	245
HAT	207	N N A H H C G G S L I N N M W I L T A A H C F R S N S N P R D W I A T	241
DESC1	246	F G V T I K P S K M K R G L R R I V E K Y K H P S H D Y D I S L A	280
HAT	242	S G I S T T F P K L R M R V R N I L I H N N Y K S A T H E N D I A L V	276
DESC1	281	E L S S P V P Y T N A V H R V C L P D A S Y E F O P G D V M F V T G F	315
HAT	277	R L E N S M T F T K D I H S V C L P A A T Q N I P P G S T A Y V P G W	311
DESC1	316	G A L K N D G Y S Q N H L R O A Q V T L T D A T T C N E P Q A V N D A	350
HAT	312	G A Q E Y A G H T V P E L R Q G C V R I I S N D V G N A P H S Y N G A	346
DESC1	351	I T P R M L C A G S L E G K T D A C G G D S G C P L V S S D A R D I W	385
HAT	347	I L S G M L C A G V P Q G G V D A C G G D S G C P L V Q E D S R R L W	381
DESC1	386	Y L A G I V S S G D E C A K P N K P G V Y T R V T A L R O W T T S K T	420
HAT	382	F I V G I V S W G D Q C G L P D K P G V Y T R V T A Y L D W I R Q Q T	416
DESC1	421	G P I	422
HAT	417	G T I	418

Figure 2

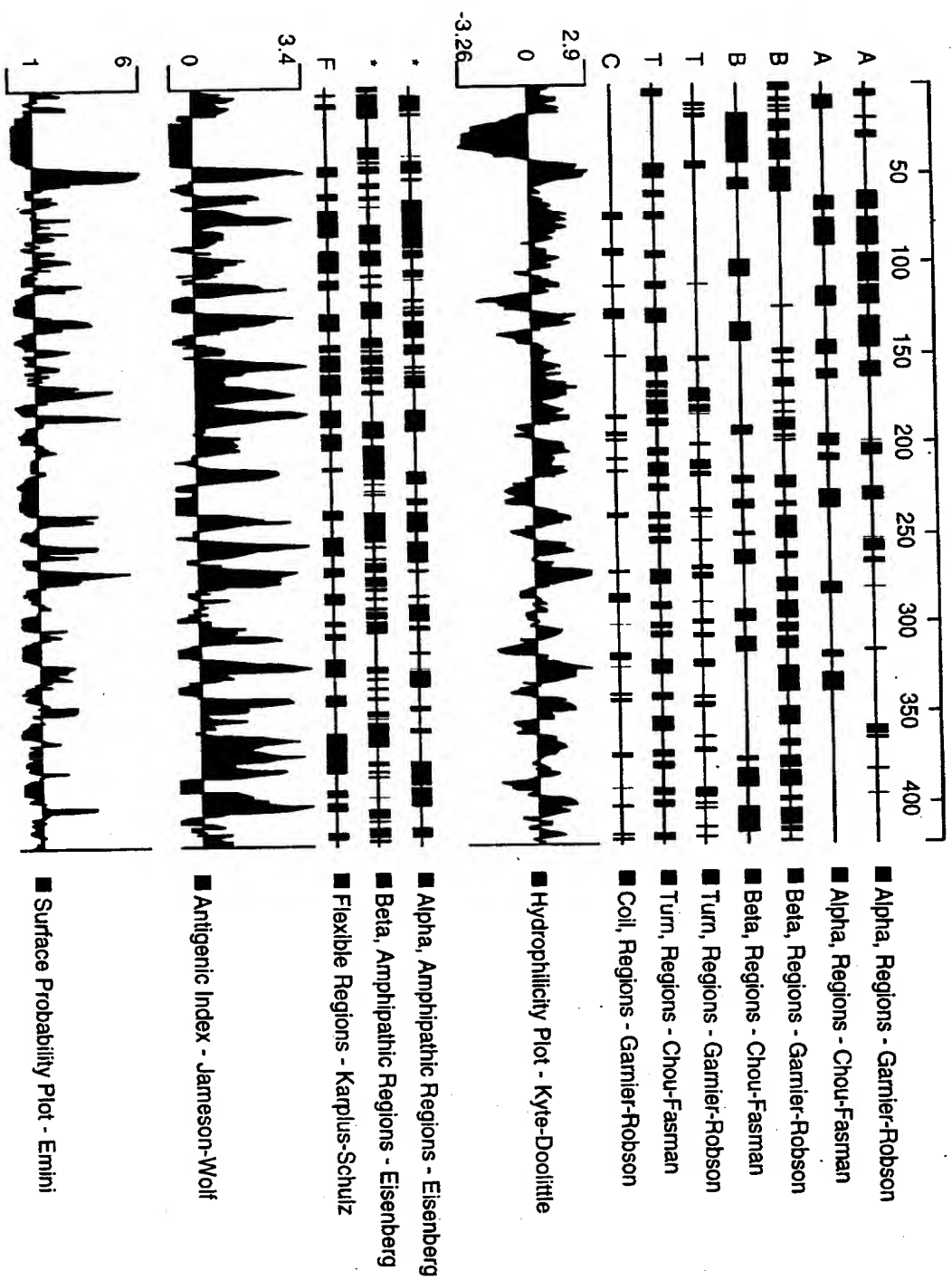


Figure 3  
 00163747.0026099



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